

*Wish you all the very best on your pursuit of the most valuable treasure*



# *RNAi screening for fat regulatory genes with SRS microscopy*

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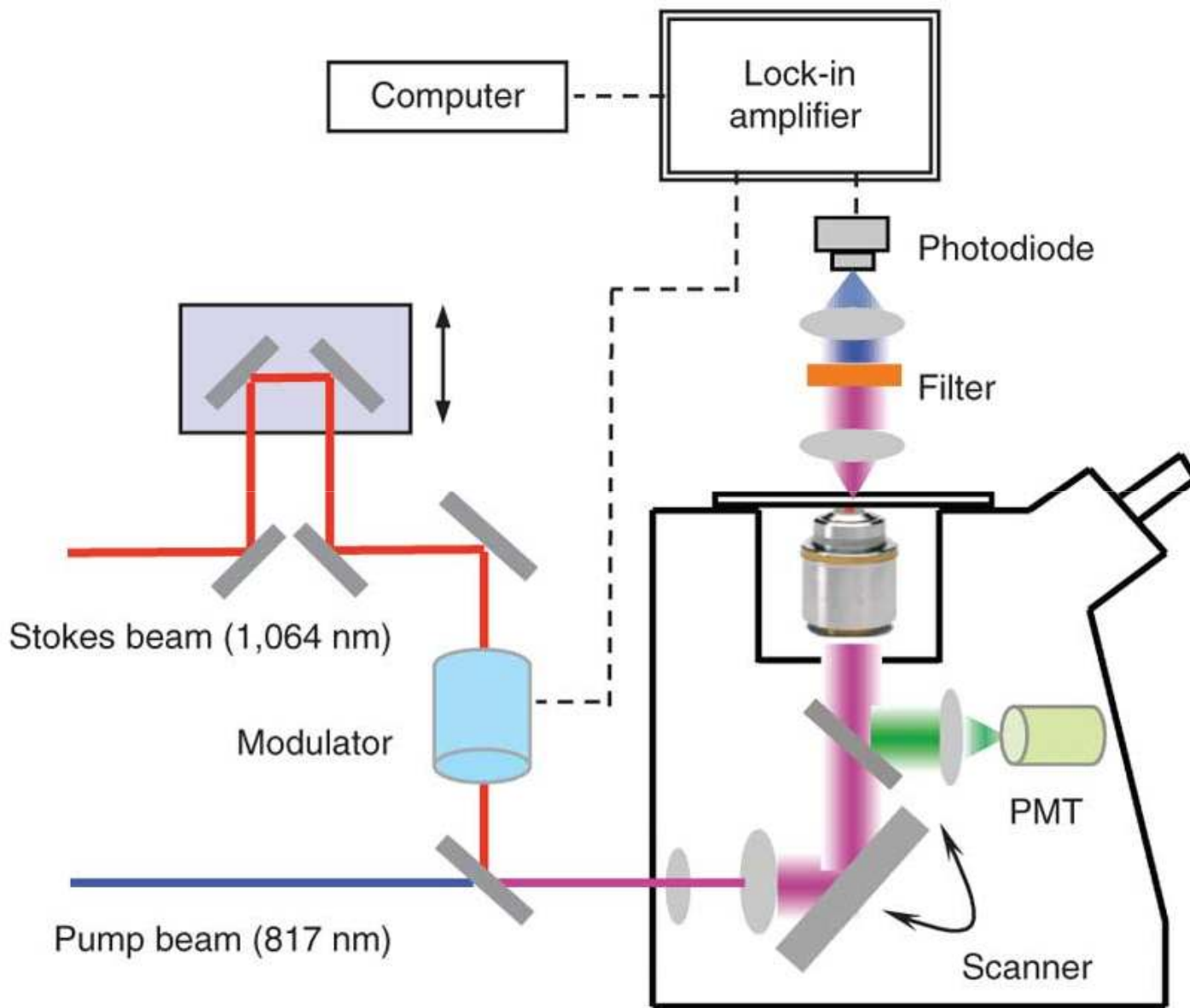
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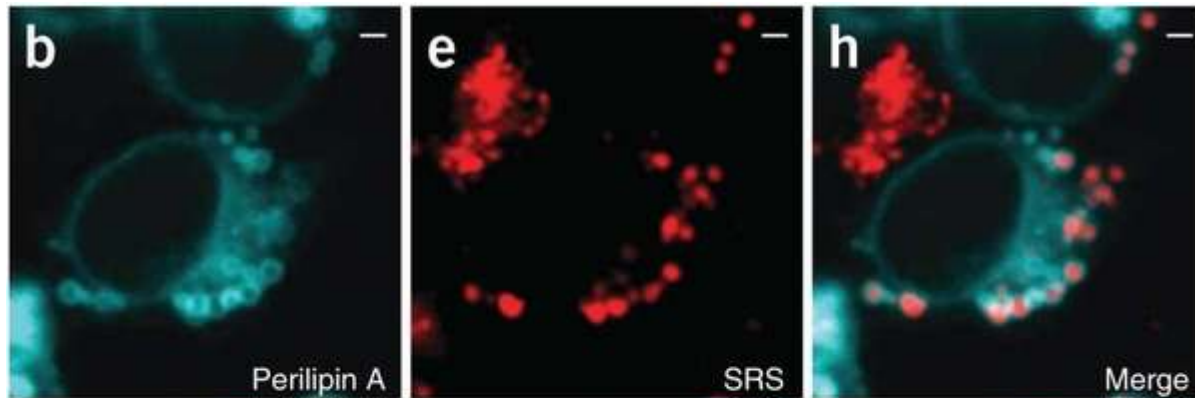
## *Back ground*

- Identification of genes regulating fat accumulation is important for basic and medical research
- obesity has reached epidemic proportions globally/ major factor for chronic diseases – type-2 diabetes, cardiovascular diseases and hypertension
- genomic screening for fat-storage modulators has been carried out in several single cellular and multicellular organisms.
- *Caenorhabditis elegans* – multicellular organism – small size, well developed genetic tools, easy to handle
- Lipid analysis in *C. elegans* has been performed by traditional biochemical approaches, fixation and staining, fluorescence imaging of live worms(vital dye feeding) and label –free coherent anti-stokes Raman scattering (CARS)

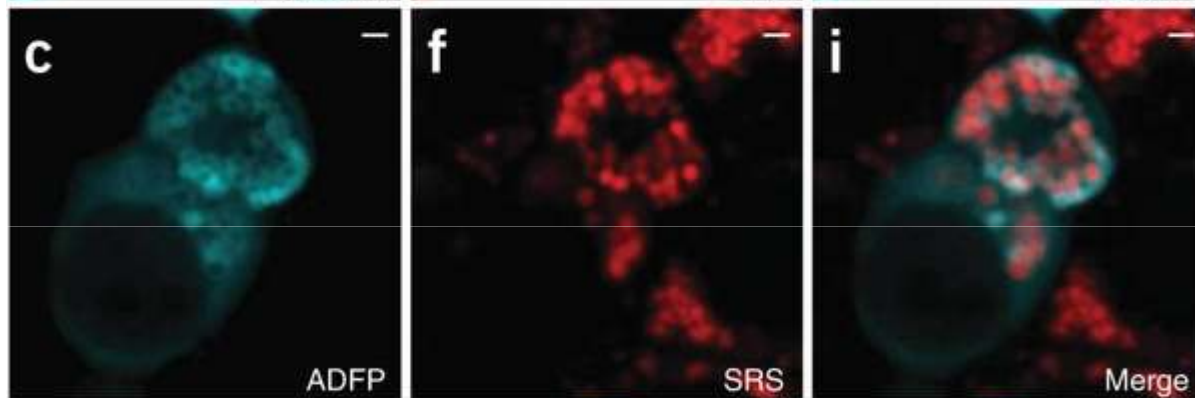
- Biochemical assays require extraction of lipids from more than 5000 worms and subsequent time consuming chromatography analysis
- The organic solvents used in fixation protocol often disrupt and interfere with structure of lipid storage compartments
- Analysis after feeding with vital dyes (Nile Red & boron-dipyromethane(BODIPY)) is complicated by unpredictable dye-incorporation efficiency – non linear relationship between observed signal and lipid quantity
- CARS signals exhibit non resonant background that limits detection sensitivity – nonlinear relationship with analyte concentration
  
- Stimulated Raman scattering (SRS) microscopy can be used to excite and detect the CH<sub>2</sub> stretching vibration of fatty acid chains – label free 3D lipid visualization



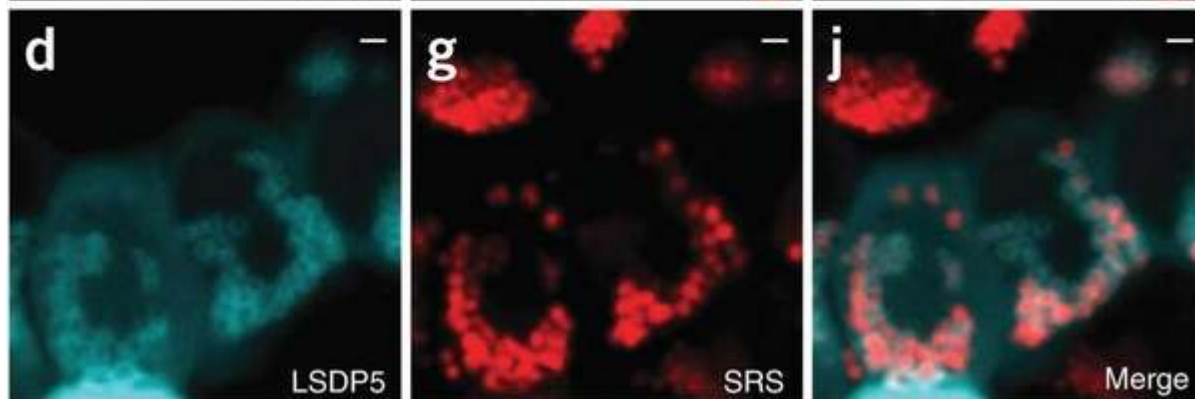
Perilipin A



ADFP

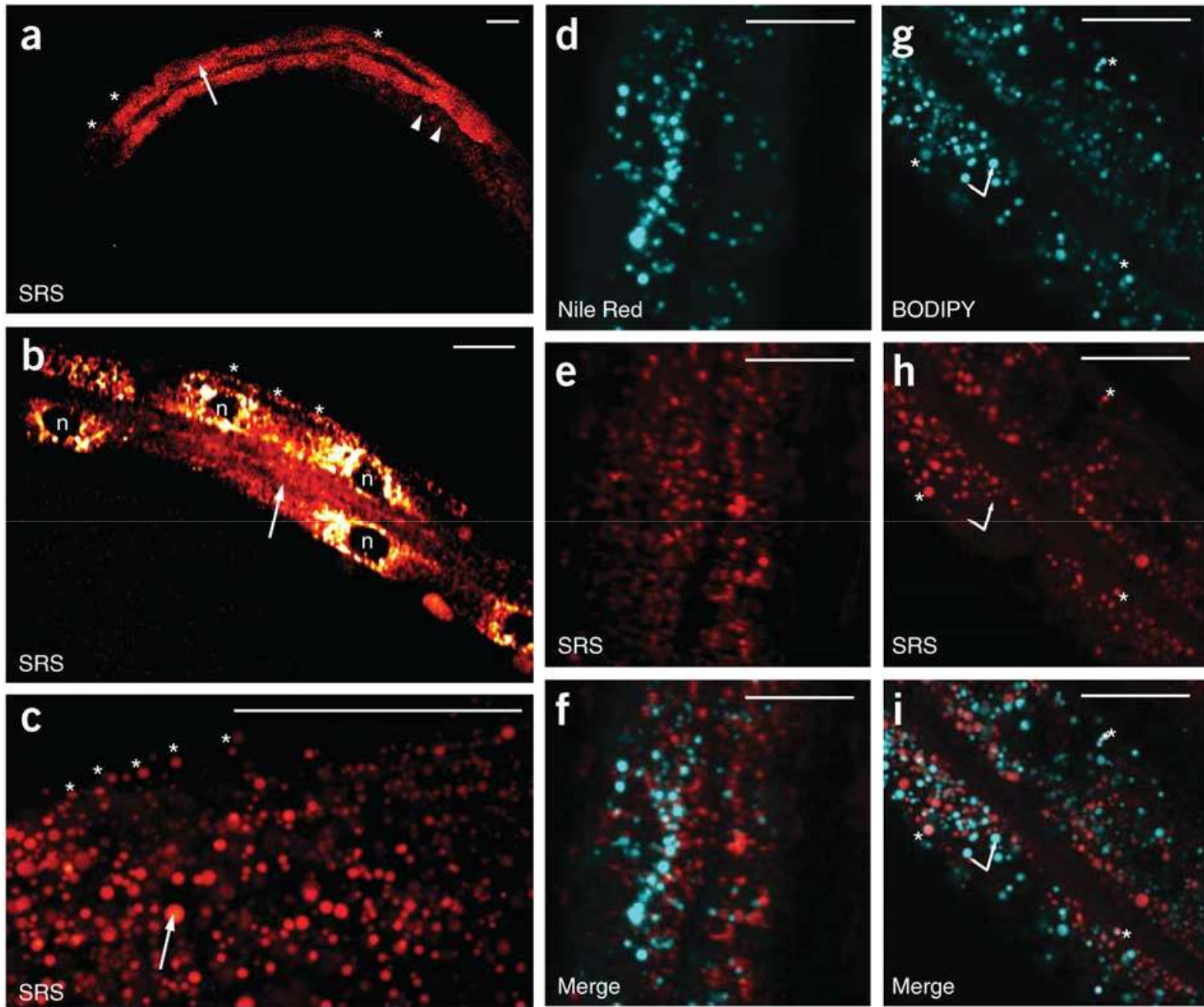


LSDP5

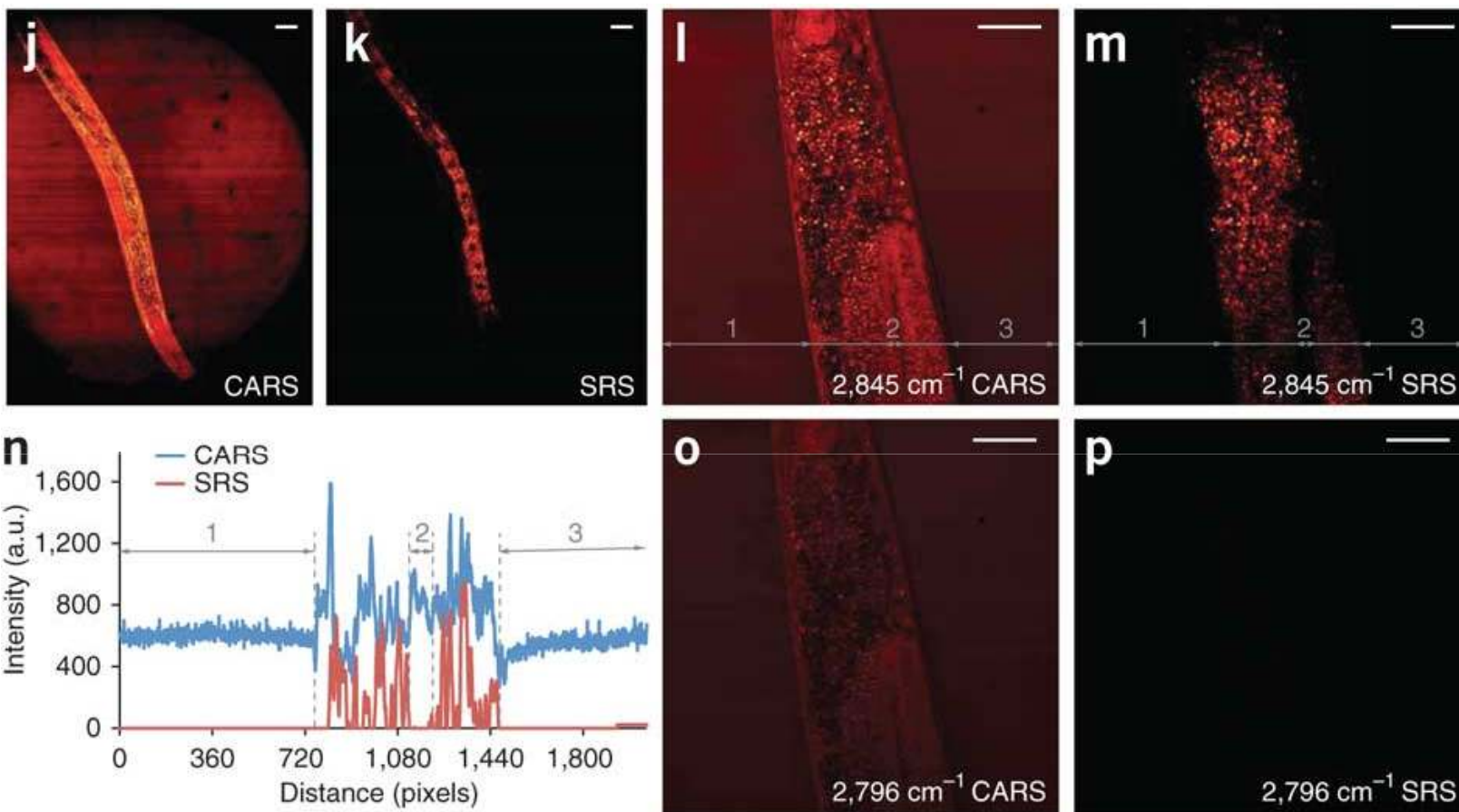


HEK -293 cells

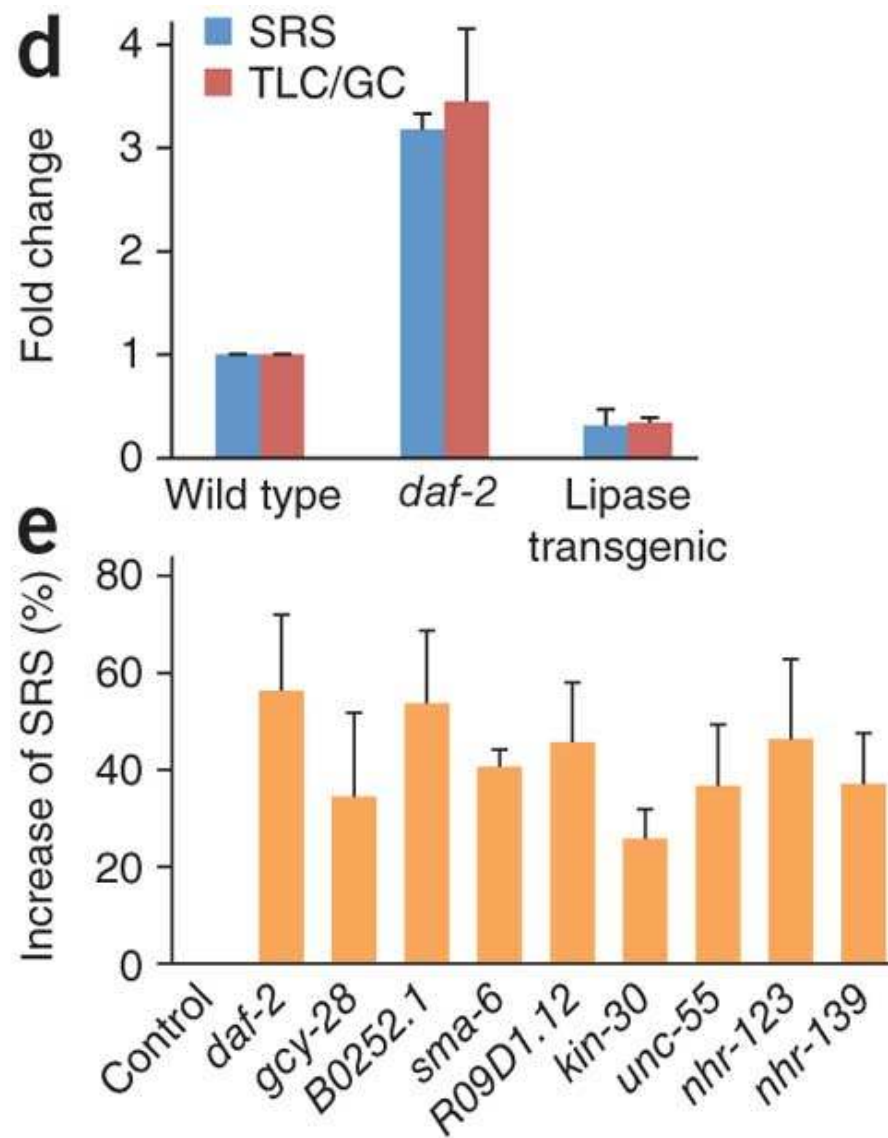
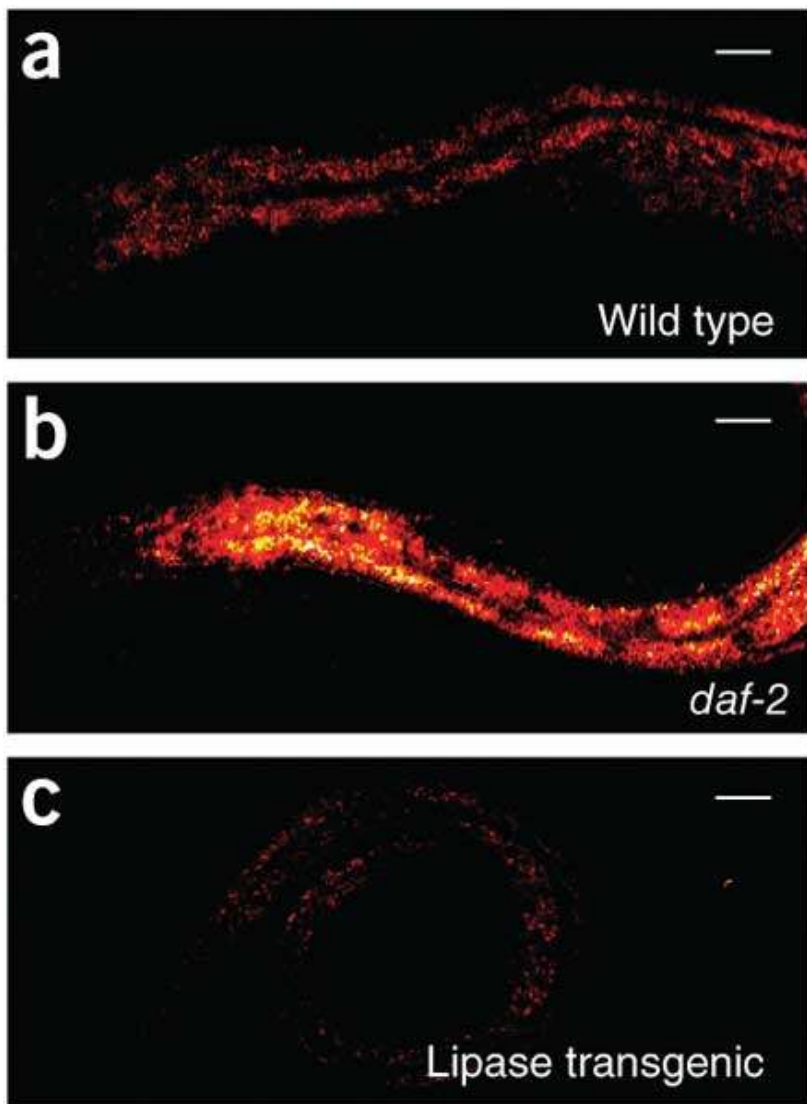
1 micro

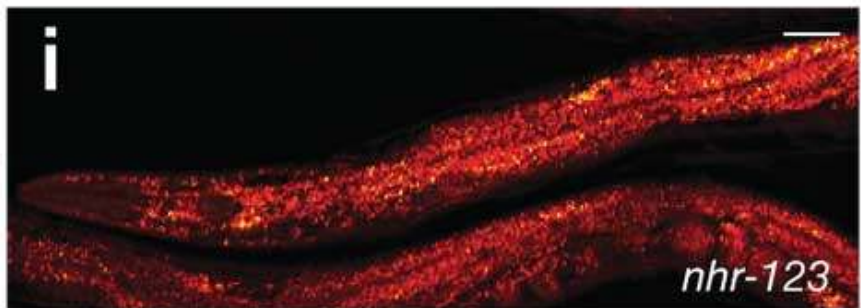
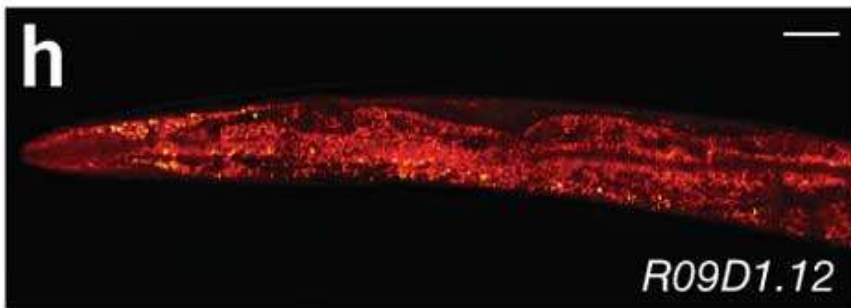
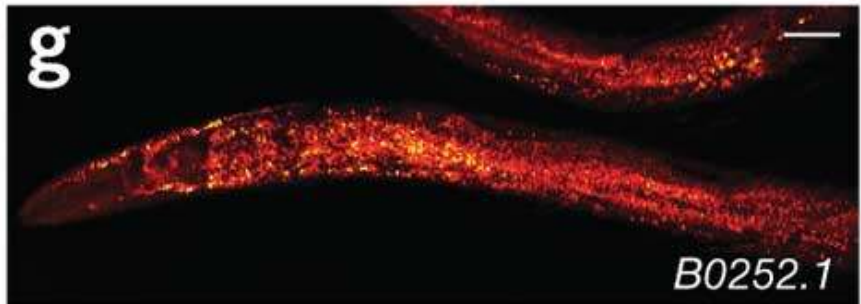
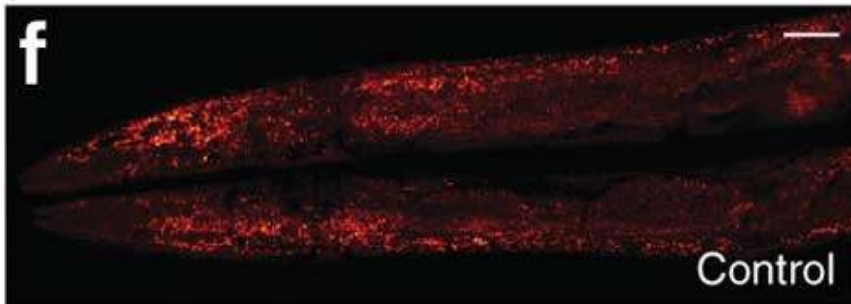


*C. elegans*









**Supplementary Table 1. Summary of newly identified genes responsible for fat storage.**

<b>Gene</b>	<b>Brief Description</b>	<b>Expression Pattern</b>	<b>Human</b>	<b>SRS Increase <math>\pm</math> STD</b>	<b>p value</b>
<i>daf-2</i>	Insulin/IGF-1 receptor	Neuron, Muscle, Intestine, etc	Yes	0.56 $\pm$ 0.16	<0.0001
<i>gcy-28</i>	Natriuretic peptide receptor	Neuron, Muscle, Intestine, etc	Yes	0.34 $\pm$ 0.17	<0.0001
<i>B0252.1</i>	FGF receptor	Unknown	Yes	0.54 $\pm$ 0.15	<0.0001
<i>sma-6</i>	Type I TGF-beta receptors	Hypodermis, Intestine, Muscle, etc	Yes	0.41 $\pm$ 0.03	<0.0001
<i>R09D1.12</i>	Protein kinase	Unknown	Yes	0.46 $\pm$ 0.12	<0.0001
<i>kin-30</i>	Tyrosine Kinase	Unknown	Yes	0.26 $\pm$ 0.06	<0.0001
<i>unc-55</i>	COUP transcription factor	Neuron, Muscle	Yes	0.37 $\pm$ 0.13	<0.0001
<i>nhr-123</i>	nuclear hormone receptor	Neuron, Intestine, Pharynx	Yes	0.46 $\pm$ 0.16	<0.0001
<i>nhr-139</i>	nuclear hormone receptor	Unknown	Yes	0.37 $\pm$ 0.10	<0.0001
<i>nhr-74</i>	nuclear hormone receptor	Seam cells	Yes	0.21 $\pm$ 0.10	0.001

*Thank you all*

*Robin John*

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